

Description of a New Species of *Amolops* Cope, 1865 (Amphibia: Ranidae) from Nepal and Nomenclatural Validation of *Amolops nepalicus* Yang, 1991

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Abstract A new species, *Amolops mahabharatensis* sp. nov., is described from Mahabharat range (middle mountain) of Nepal. The new species is a member of the *A. marmoratus* group and is distinguished from its congeners using morphological and molecular data. The new species is diagnosed by having numerous warts on dorsum and flanks, dorsal body greyish olive-brown/light brown, presence of vomerine teeth series, presence of a pair of subgular vocal sacs in males and tadpole with III:5+5/1+1:II – labial tooth row. In addition, evidenced by the phylogenetic and morphological analyses in this study, *Amolops nepalicus* is shown to be a valid species. A molecular phylogenetic analysis based on 16S ribosomal gene for six members of the *A. marmoratus* group suggested that the new species is sister to a clade containing *A. nepalicus* and *A. panhai*. The uncorrected genetic divergences between the new species and its closest congeners *A. nepalicus*, *A. panhai* and *A. marmoratus* based on 16S rRNA gene were 10.5%, 11.6%, and 8.6% respectively. The new species showed wide distribution in the Mahabharat range of Nepal.

Keywords *A. marmoratus* group, mahabharat range, molecular phylogeny, taxonomy

1. Introduction

Frogs of the genus *Amolops* (Cope, 1865) are renowned for their extraordinary diversity in fast flowing streams in South and Southeast Asia (Frost, 2019). Most species of *Amolops* are habitat specialist (Khatiwada and Haugaasen, 2015) and might have relatively small and mostly allopatric distributions in this range. This genus is comprised of 60 currently recognized species (Frost, 2019). *Amolops* are highly adapted for torrent habitat conditions (Schleich and Kästle, 2002); they possess several morphological modifications such as the presence of adhesive disks on the tips of the digits with circummarginal grooves (Biju *et al.*, 2010) and tadpoles with gastromyzophorus adhesive disk (Altig and McDiarmid, 1999). In the recent five years, more than 10 new species of *Amolops* have been recorded (Sung *et al.*, 2016; Jiang *et al.*, 2016; Fei *et al.*, 2017; Chan *et al.*, 2018; Yuan *et al.*, 2018; Lyu *et al.*, 2019a, 2019b; Pham *et al.*, 2019; Yu *et al.*, 2019).

Previous taxonomic and amphibian distribution studies were primarily based on morphological examination of specimens deposited in the museums (Dubois and Matsui, 1983; Nanhoie and Ouboter, 1987; Yang, 1991; Das, 1998; Shah and Tiwari, 2004). Three species of *Amolops* are currently recognized in Nepal: *A. formosus* (Günther, 1875), *A. marmoratus* (Blyth, 1855), and *A. monticola* (Anderson, 1871) (Frost, 2019; Schleich and Kästle, 2002). Apparently, *A. himalayanus* was also reported from Nepal based on a tadpole (Kripalani, 1961), which was later doubted by Schleich and Kästle (2002). Most *Amolops* populations in the Mahabharat ranges (between 400–2 000 m) of Nepal have been assigned to *A. marmoratus* (Schleich and Kästle, 2002; Shah and Tiwari, 2004), without any detailed morphological or molecular comparisons. *Amolops marmoratus* remained an unresolved and challenging species group for identification because of the

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Received: 3 October 2019 Accepted: 25 January 2020

high degree of morphological similarities among its members (Bossuyt and Milinkovitch, 2000; Dever *et al.*, 2012). For instance, Yang (1991) described *Amolops nepalicus* from Arun river basin, type locality: “Sabha, Kbota” = Sabha Khola, eastern Nepal based on adult males and tadpole morphology. Later on, Dubois (2000; 2004) revised the taxonomic status of *A. nepalicus* and synonymised it with *Amolops marmoratus*. Since the recognition on the *Amolops marmoratus* complex has been discussed recently and the true *Amolops marmoratus* is only recognized from southeastern Myanmar (Dever *et al.*, 2012; Lyu *et al.*, 2019b), the nomenclatural validation of *A. nepalicus* and the taxonomic status on other Nepali populations previously assigned to *A. marmoratus* need to be clarified.

Although a number of molecular studies on *Amolops* have been published on Southeast Asian species (Jiang *et al.*, 2016; Sung *et al.*, 2016; Lyu *et al.*, 2019a), no integrative taxonomic studies have been used to evaluate the taxonomic position of *Amolops* species in Nepal. During field surveys in 2014 to 2018 in the eastern, central and western Nepal, we collected a series of adult specimens and tadpoles of *Amolops* frogs. Detailed

morphological and molecular analyses suggested that the *Amolops* population from middle mountain region of Nepal (Mahabharat range) are genetically and morphologically distinct from previously known species of genus the *Amolops*. Thus, we provide a description of the Mahabharat range population of *Amolops* as a new species. Besides, this study also found that the *Amolops* frogs collected from Dobhan population was genetically distinct with other members of genus *Amolops* and showed morphological similarities with *Amolops nepalicus* and further discuss the nomenclatural validation of *A. nepalicus*.

2. Materials and Methods

2.1. Sampling and specimen collection Field work was carried out from May to September each year from 2014 to 2018 in the eastern, central and western Nepal (Figure 1). Following the methods of (Khatiwada *et al.*, 2019a), amphibians were surveyed using nocturnal time constrained visual encounter surveys between 19:00 h–00:00 h. This method involved systematic walking at a slow pace, intensively searching for amphibian

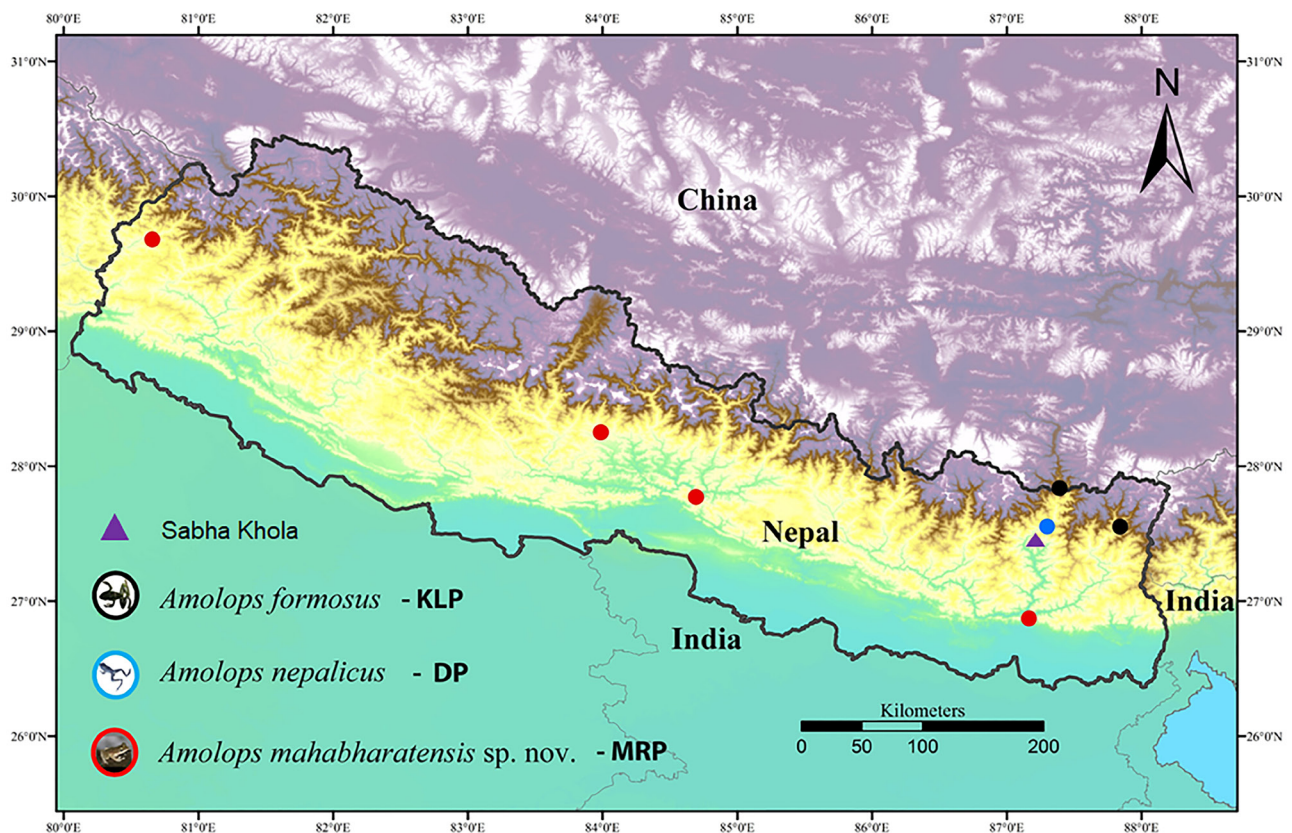


Figure 1 Location of the study area showing the sampling localities in Nepal. Purple triangle refers to Sabha Khola-type locality of *Amolops nepalicus*, red dots refer to sampling locations of *Amolops mahabharatensis* sp. nov. (MRP), blue dot refers to sampling location of *Amolops nepalicus* (DP) and black dots refer to sampling sites of *Amolops formosus* (KLP).

species by turning over stones, logs, leaf litter, tree branches, shrubs and bushes along the transects using both visual and acoustic aids. Specimens were captured by hand, photographed and were euthanized using 20% Benzocaine gel, fixed in 4% formalin for 24 hours and preserved in 75% ethanol. Tadpoles at different developmental stages were also sampled and were preserved in 70% alcohol. Sex was determined by the observation of secondary sexual characteristics, such as vocal sacs and nuptial pads in males. Females were identified by direct inspection of the enlargement of the coelomic cavity and checked the presence of ovary and eggs by minor dissections. Tissue samples were taken from thigh muscle in adults and small portion of tail muscle of tadpoles and preserved in 95% ethanol for further molecular analysis. A total of 33 individuals (one individual from Lamatar, one from Kimathanka, 12 from Dobhan, two from Barahakshetra, 10 from Hattibang, three from Pokhara and three individuals from Latinath, Darchula (for details see Molecular methods section) were included in the morphological examination. All morphological measurements were taken from the preserved specimens. Handling and processing of all amphibians were in accordance with the guidelines of the Department of National Park and Wildlife Conservation, Government of Nepal and Animal Care and Use Committee of Chengdu Institute of Biology, Chinese Academy of Sciences. Vouchers were deposited at the Museum collection of Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

2.2 Morphological measurements Morphological measurements were carried out using digital calliper (to nearest 0.01 mm). Measurements descriptions of adults and tadpoles are provided in Table 1 as described by (Khatiwada *et al.*, 2019a). Comparative morphological data of species: *A. marmoratus* was taken from Dever *et al.* (2012) whereas *A. nepalicus*, *A. monticola* and *A. formosus* were taken from their original descriptions Yang (1991), Anderson (1871) and Günther (1875) respectively. Preserved *Amolops* specimens were also measured in the herpetological museum of Chengdu Institute of Biology (CIB), Chinese Academy of Sciences, Chengdu, China, Central Department of Zoology, Tribhuvan University (CDZ-TU) and the British Museum of Natural History (BMNH), UK. A total of 13 *Amolops* species were examined (Appendix Table S1).

2.3. Molecular methods Total genomic extraction was carried out with thigh muscle preserved in 95% ethanol using the DNeasy Tissue Kit (QIAGEN). The mitochondrial gene 16S ribosomal RNA gene (hereafter 16S) was amplified and subjected to polymerase chain reaction (PCR) with primers as described by Matsui *et al.* (2006). The amplified PCR products were purified using Qiagen PCR purification kit and sequences were obtained from an ABI 3100 automated sequencer.

All sequences were deposited in GenBank under accession numbers (GenBank accession numbers are available in Table 2). Available nucleotide sequences of 16S gene of *Amolops* were downloaded from NCBI GenBank database and aligned with ClustalW built into BIOEDIT Version 7.1.9 (Thompson *et al.*, 1997) using the default parameters. *Rana dalmatina* and *Hylarana leptoglossa* were selected as the outgroups (Stuart *et al.*, 2010). Alignments were also checked and manually edited, if necessary. Maximum Likelihood (ML) analysis was conducted with the rapid bootstrapping algorithm using the program RAXML v8.00 (Stamatakis, 2014) on the CIPRES Science Gateway server v3.2 (Miller *et al.*, 2010). Nodal support for ML was assessed with 1000 rapid bootstrap replicates (BS). The Bayesian inference (BI) analyses and the best-fit substitution model were selected under the Bayesian Information Criterion by the program jModeltest 2.1.4 (Darriba *et al.* 2012). The best-fit substitution model for the 16s dataset was GTR + I + G. BI analyses were conducted in the program MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Two independent runs were initiated each with four simultaneous Markov Chain Monte Carlo (MCMC) chains for 20 million generations and sampled every 1000 generations. The convergence of chains and burn-in period of all runs were examined by plots of log-likelihood scores and low standard deviation of split frequencies. The first 25% generations were discarded as burn-in, and the last remaining trees were used to create a 50% majority-rule consensus tree and estimate Bayesian posterior probabilities (BPP). The graphical viewer Figtree (Rambaut, 2007) was used to edit the resulting output of RAXML and MrBayes analyses. Pairwise divergences (uncorrected p-distance) between species on 16s dataset were calculated using MEGA 7 (Kumar *et al.*, 2016).

2.4. Statistical analysis Principal Component Analysis (PCA) was used to show the overall morphological variation between the species. Prior to PCA, all morphometric data were converted into ratio values of SVL (morphological character/SVL*100) to reduce allometric bias. Statistical analysis was performed in R package vegan (Oksanen *et al.*, 2016) in R-software version 3.3.0 (R Development Core Team, 2016).

3. Results

Molecular analyses

The aligned dataset of 16S contained 620 bps including 409 variable sites and 111 parsimony informative sites (excluding outgroups). The maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees constructed from 16S DNA sequences produced identical topologies with strong node support (Figure 2). The phylogenetic analysis suggested that the *Amolops* specimens (including tadpoles from Hattibang) from

Table 1 Morphological characters used and their measurement descriptions for adults and tadpoles.

Abbreviation	Morphology	Measurement description
SVL	Snout vent length	From tip of snout to posterior edge of vent
HL	Head length	From angle of jaws and snout-tip
HW	Head width	Measured at the posterior angle of the jaws
SL	Snout length	From tip of snout to the anterior corner of eye
ED	Eye diameter	Horizontal diameter of the eye
IND	Internarial distance	Minimum distance between the external nares
ENL	Eyelid-naris distance	Minimum distance between eyelid and rim of naris
UEW	Upper eyelid width	Greatest width of upper eyelid
IOW	Inter orbital width	Minimum distance between upper eyelids
TYD	Tympanum diameter	Largest tympanum diameter
TYE	Tympanum orbit distance	Distance from anterior border of tympanum to posterior orbital border
FAL	Forearm length	Distance from the elbow to the base of the outer metacarpal tubercle
HAL	Length of hand	Measured from the base of the outer metacarpal tubercle to the tip of the third finger
F1	First finger length	Distance from the base of the second finger to the tip of the first finger
F2	Second finger length	Distance from the base of the first finger to the tip of the second finger
F3	Third finger length	Distance from the base of the second finger to the tip of the third finger
F4	Fourth finger length	Distance from the base of the third finger to the tip of the fourth finger
THL	Thigh length	Distance from groin to keen
SHL	Shank length	Distance from keen to heel
TL	Tarsus length	Distance from heel to inner metatarsal tubercle
FOL	Foot length	Distance from inner metatarsal tubercle to the tip of the fourth toe
T1	First toe length	Maximum length from the base of the first subarticular tubercle first toe tip
T2	Second toe length	Maximum length from the base of the first subarticular tubercle second toe tip
T3	Third toe length	Maximum length from the base of the first subarticular tubercle third toe tip
T4	Fourth toe length	Maximum length from the base of the first subarticular tubercle fourth toe tip
T5	Fifth toe length	Maximum length from the base of the first subarticular tubercle fifth toe tip
Tadpole morphology		
TL	Total length	Distance from the tip of the snout to the tip of the tail.
BL	Body length	Distance from the tip of the snout to the junction of the posterior body and the tail musculature.
TAL	Tail length	Distance from the junction of the posterior body and the tail musculature to the tip of the tail.
BW	Body width	The highest body width.
BH	Body height	The highest body height.
HW	Head width	Measured at the posterior angle of the jaws
TMH	Tail muscle height at base of tail	Maximum tail muscle height
UTF	Upper tail fin height	The maximum height of the upper fin, from the upper margin of the tail musculature to the upper margin of the upper fin.
LTF	Lower tail fin height	The maximum height of the lower fin, from the lower margin of the lower fin to the lower margin of the tail musculature
TMW	Tail muscle width at base of tail	Maximum tail muscle width
MTH	Maximum height of tail	The maximum height of the tail.
ENL	Eyelid-naris distance	Minimum distance between eyelid and rim of naris
SN	Nostril to the tip of the snout	Distance from the nostril to the tip of the snout
SSD	Snout-spiracle distance	From the tip of the snout to the posterior margin of the spiracle.
IND	Internarial distance	Minimum distance between the external nares
IOW	Inter orbital width	Minimum distance between upper eyelids
ED	Eye diameter	Horizontal diameter of the eye

The uncorrected genetic divergence between MRP and DP was 13%, with MRP and topotypic *A. marmoratus* was 12% and MRP and *A. panhai* was 15% (Table 3). *Amolops* species from DP was sister taxon of *A. panhai* and the uncorrected genetic divergence between species of DP and *A. panhai* was 15%



Table 2 Samples used in molecular analysis.

Sample ID	Voucher No.	Species	Locality	Genebank accession No. (16s)
1	CDZMTU0110	<i>Amolops mahabharatensis</i> sp. nov.	Hattibang, Chitwan district, Nepal	MT124507
2	CDZMTU0111	<i>Amolops mahabharatensis</i> sp. nov.	Hattibang, Chitwan district, Nepal	MT124508
3	CDZMTU0112	<i>Amolops mahabharatensis</i> sp. nov.	Hattibang, Chitwan district, Nepal	MT124509
4	CDZMTU0113	<i>Amolops mahabharatensis</i> sp. nov.	Hattibang, Chitwan district, Nepal	MT124510
5	CDZMTU0114	<i>Amolops mahabharatensis</i> sp. nov.	Pokhara, Kaski district, Nepal	MT124511
6	CDZMTU0115	<i>Amolops mahabharatensis</i> sp. nov.	Pokhara, Kaski district, Nepal	MT124512
7	CDZMTU0116	<i>Amolops mahabharatensis</i> sp. nov.	Barahakshetra, Sunsari district, Nepal	MT124513
8	CDZMTU0117	<i>Amolops mahabharatensis</i> sp. nov.	Barahakshetra, Sunsari district, Nepal	MT124514
9	CDZMTU0118	<i>Amolops mahabharatensis</i> sp. nov.	Latinath, Darchula district, Nepal	MT124515
10	CDZMTU0119	<i>Amolops mahabharatensis</i> sp. nov.	Latinath, Darchula district, Nepal	MT124516
11	CAS232997	<i>A. afganus</i>	Kachin, Myanmar	JF794431
12	SYSa003452	<i>A. albispinus</i>	Guangdong, China	KX507312
13	LSUHC7673	<i>A. australis</i>	Johor, Malaysia	MF061745
14	CAS 233991	<i>A. bellulus</i>	Yunnan, China	FJ417127
15	SYSa7512	<i>A. chayuensis</i>	Xizang, China	MK604877
16	SYSa003136	<i>A. chunganensis</i>	Sichuan, China	KX507310
17	ROM14528	<i>A. cremnobatus</i>	Khe Moi, Vietnam	DQ204477
18	AMNH168727	<i>A. cucae</i>	Van Ban, Vietnam	FJ417144
19	SYSa001737	<i>A. daiyunensis</i>	Mt. Daiyu, Fujian, China	KX507306
20	CDZMTU0145	<i>A. formosus</i>	Kima thanka, Sankhuwasabha, Nepal	MT124517
21	CDZMTU0146	<i>A. formosus</i>	Kima thanka, Sankhuwasabha, Nepal	MT124518
22	CDZMTU0147	<i>A. formosus</i>	Lamatar, Taplejung, Nepal	MT124519
23	CDZMTU0148	<i>A. formosus</i>	Lamatar, Taplejung, Nepal	MT124520
24	CKO574	<i>A. gerutu</i>	Perak, Malaysia	MF061741
25	C93161	<i>A. lifanensis</i>	Sichuan, China	AB211481
26	SYSa004580	<i>A. hainanensis</i>	Hainan, China	KX507320
27	SYSa004577	<i>A. hongkongensis</i>	Hong Kong, China	KX507317
28	CAS216496	<i>A. indoburmanensis</i>	Rakhine, Myanmar	JF794460
29	SCUM050435CHX	<i>A. jinjiangensis</i>	Deqing, China	EF453741
30	SCUM045822HX	<i>A. xinduoqiao</i>	Sichuan, China	EF453742
31	C93150	<i>A. lifanensis</i>	Sichuan, China	AB211482
32	CI8	<i>A. loloensis</i>	Sichuan, China	AB211478
33	SCUM045807HX	<i>A. loloensis</i>	Sichuan, China	EF453743
34	C62	<i>A. mantzorum</i>	Sichuan, China	AB211479
35	CAS240594	<i>A. marmoratus</i>	Mon, Myanmar	JF794453
36	CAS240591	<i>A. marmoratus</i>	Mon, Myanmar	JF794454
37	SYSa006657	<i>A. medogensis</i>	Xizang, China	MK573813
38	1999.5811	<i>A. mengyangensis</i>	Lao Cai, Sapa, Vietnam	KR827703
39	CDZMTU0133	<i>A. nepalicus</i>	Dobhan, Sankhuwasabha, Nepal	MT124521
40	CDZMTU0134	<i>A. nepalicus</i>	Dobhan, Sankhuwasabha, Nepal	MT124522

(Continued Table 2)

Sample ID	Voucher No.	Species	Locality	Genebank accession No. (l6s)
41	CDZMTU0135	<i>A. nepalicus</i>	Dobhan, Sankhuwasabha, Nepal	MT124523
42	CDZMTU0137	<i>A. nepalicus</i>	Dobhan, Sankhuwasabha, Nepal	MT124524
43	SYSa007508	<i>A. nyingchiensis</i>	Xizang, China	MK573815
44	KUHE20133	<i>A. panhai</i>	Phetchaburi, Thailand	AB211488
45	CAS229816	<i>A. panhai</i>	Tanintharyi, Myanmar	JF794451
46	ROM26365	<i>A. ricketti</i>	Shanghang, Fujian, China	DQ204486
47	SYSa4165	<i>A. sinensis</i>	Guangdong, China	MK263262
48	ROM 7555	<i>A. spinapectoralis</i>	Gia Lai, Vietnam	AF206456
49	SCUM050253YJ	<i>A. torrentis</i>	Hainan, China	EF453744
50	SYSa003931	<i>A. tuberodepressus</i>	Yunnan, China	MG991904
51	C-green05	<i>A. viridimaculatus</i>	Yunnan, China	AB211480
52	SYSa001716	<i>A. wuyiensis</i>	Fujian, China	KX507304
53	SYSa4165	<i>A. yatseni</i>	Guangdong, China	MK263262
54	SYSa003452	<i>A. yunkaiensis</i>	Yunkaishan, China	KX507312
55	IABHU3784	<i>Hylarana leptoglossa</i>	Bangladesh	AB530528
56	51331	<i>Rana dalmatina</i>	Germany	AY147941

whereas with *A. marmoratus* it was divergent by 13% (Table 3). KLP formed a distinct lineage in the BI analysis. The genetic distance between KLP with MRP and DP were 13% and 15% respectively.

Morphological analysis

Based on phylogenetic analysis, MRP was the sister taxon to species from DP. The result from PCA based on size-corrected values support the molecular analyses and showed the overall morphological variation between MRP, DP and KLP. For the male population, PCA extracted seven principal component axes with eigenvalues greater than one where the first two component explained 55.4% of the total variation (Table 4). The first two principal component axes radially separated MRP from DP based on hand, finger and toe length (Figure 3) in male population. Species with larger and positive scores on PC1 reflected shorter head length, head width, snout length, inter-orbital width, length of 1st, 2nd, 3rd and 4th fingers, length of femur, tibia, tarsus and meta-tarsus, length of 1st, 2nd, 3rd, 4th and 5th toes while a negative scores signified larger eye diameter, width of upper eyelid, inter-narial width, tympanum diameter, length of arm and hand. The second PC with negative scores were associated with species having larger head length, internarial width, length of arm, hand and tarsus whereas positive scores were associated with species with shorter morphological traits such as head width, snout length, eye diameter, nostril to eye, width of upper eyelid, inter-orbital width, tympanum diameter, length of 1st, 2nd, 3rd and 4th fingers, length of femur, tibia and meta-tarsus, length of 1st, 2nd,

3rd, 4th and 5th toes. For females, PCA extracted six principal component axes with eigenvalues greater than one and the first two components explained 56.3% of the total variation (Table 4). The first two principal component axes radially separated the female populations of MRP, DP and KLP based on length forelimbs fingers, tibia, meta-tarsus and toes (Table 4).

Table 4 Taxonomic review Based on phylogenetic and morphological results, *Amolops* populations from Nepal (MRP, DP and KLP) represented three distinct species. The results confirmed that there is substantial genetic divergence between the specimens from Mahabharat Range Population (MRP) and other known *Amolops* species, and support that this population is a distinctly evolving lineage, representing an undescribed species. This population also presents distinct morphological characteristics that are not observed in the closely related species *A. marmoratus*, *A. panhai* and *A. nepalicus*.

The Dobhan Population (DP) showed the large genetic and morphological variation from MRP and *A. marmoratus* and possessed similar morphological features with *A. nepalicus* as described by Yang (1991). Hence, the population of the Dobhan lineage has been identified as *A. nepalicus*. Additionally, the geographical distance between topotypic *A. nepalicus* (Shaba Khola, Sankhuwasabha) and Dobhan, Sankhuwasabha population in this study is in proximity (approximately 14 km north from the type locality) which further substantiated the nomenclature validation. Therefore, the explanations provided by the Dubois (2004) are not here considered adequate justification for a synonymisation of *A. nepalicus* as a *A.*

marmoratus.

The Kimathangka Lamatar Population (KLP) population showed the large genetic and morphological variation from MRP and DP and possessed similar morphological features with *A. formosus* (holotype - BMNH 1947.2.4.18) deposited at the British Museum of Natural History (Günther 1875). Hence, the population of the KLP lineage has been identified as *A. formosus*. Taxonomic accounts

Amolops mahabharatensis sp. nov. (Figure 4 and 5)

Holotype: CDZMTU0110 (Figure 4 and 5), an adult male found resting on boulder associated with subtropical degraded forest beside wide stream (about 5 m) on Hattibang, collected from Hugdi Khola (Khola refers to stream in Nepali) Hattibang, Chitwan district, Nepal, 27.7741048°N, 84.6944107°E; elevation 775 m a.s.l. collected by Janak Raj Khatiwada between 19:30 and 20:00 h on 16th July 2016 and deposited in the collection of

Central Department of Zoology, Tribuvan University, Kirtipur, Kathmandu, Nepal.

Suggested common name: Mahabharat Torrent frog

Suggested common name in Nepali: पाहाडी पिर्रे पाहा (Pahadi Pirre Paha)

Etymology: The species name is derived from the noun “Mahabharat” which refers to the foothills of Himalaya, from where this species were collected.

Paratypes: CDZMTU0114 (adult female – Figure 6), CDZMTU0115 (adult female) and CDZMTU0111 – 0113 (adult male) from the same location as the holotype and deposited in the collection of Central Department of Zoology, Tribuvan University, Kirtipur, Kathmandu, Nepal.

Diagnosis: The new species is assigned to genus *Amolops* based on the molecular and morphological similarity (first finger with circummarginal and transverse grooves on disk

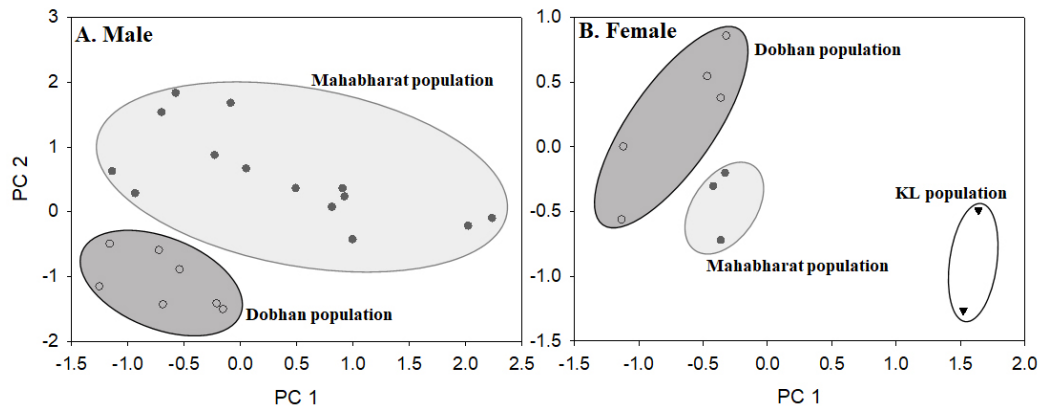


Figure 3 Principal Component Analysis (PCA) based on 24 morphometric traits. Prior to PCA, all morphometric data were converted to percentage value of SVL.

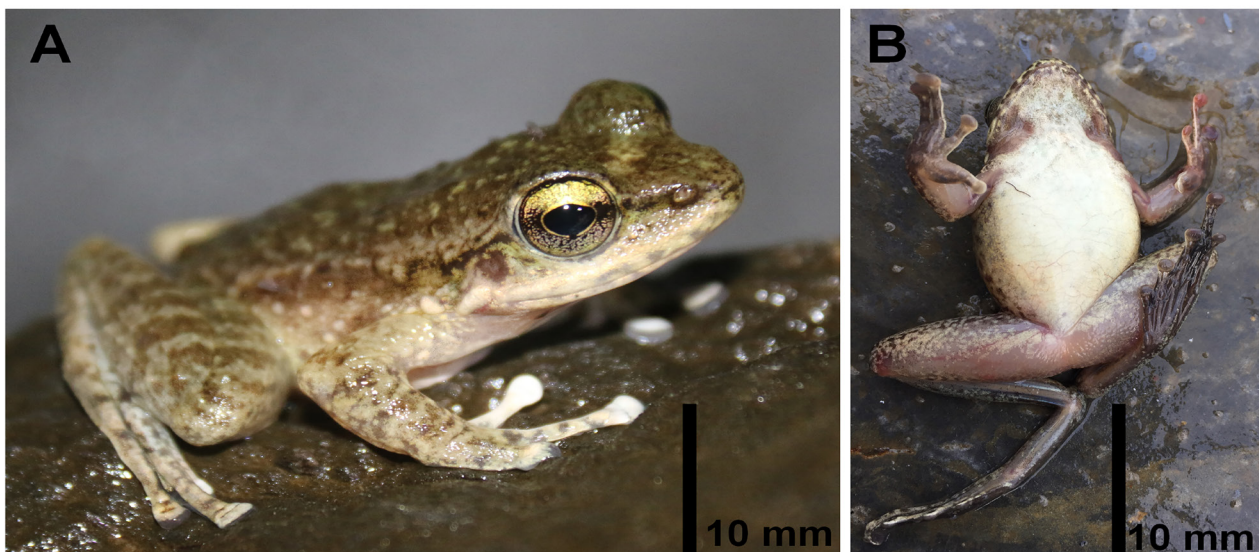


Figure 4 Male holotype in natural habitat A. Dorsolateral view B. Ventral view

Table 3 Uncorrected (*p*) pairwise distances (%) of the 16s gene sequences among species in the *A. marmoratus* and *A. monticola* group.

SN	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	MRP	0.00																				
2	DP	0.13																				
3	KLP	0.15	0.13																			
4	<i>A. panhai</i>	0.16	0.14	0.14																		
5	<i>A. marmoratus</i>	0.11	0.12	0.13	0.14																	
6	<i>A. indoburmensis</i>	0.17	0.15	0.17	0.16	0.14																
7	<i>A. chunganensis</i>	0.13	0.14	0.09	0.16	0.12	0.16															
8	<i>A. viridimaculatus</i>	0.17	0.14	0.05	0.15	0.15	0.17	0.08														
9	<i>A. medogensis</i>	0.17	0.15	0.06	0.16	0.15	0.16	0.10	0.05													
10	<i>A. nyingchiensis</i>	0.16	0.16	0.08	0.17	0.15	0.19	0.08	0.08	0.06												
11	<i>A. chayuensis</i>	0.15	0.15	0.07	0.15	0.13	0.15	0.08	0.07	0.05	0.06											
12	<i>A. afganus</i>	0.20	0.18	0.20	0.22	0.16	0.14	0.19	0.18	0.16	0.16	0.17										
13	<i>A. bellulus</i>	0.14	0.14	0.06	0.15	0.16	0.18	0.06	0.06	0.08	0.03	0.07	0.19									
14	<i>A. jinjiangensis</i>	0.15	0.13	0.06	0.14	0.13	0.15	0.07	0.05	0.07	0.06	0.04	0.16	0.04								
15	<i>A. loloensis</i>	0.16	0.13	0.06	0.14	0.14	0.15	0.07	0.05	0.08	0.06	0.04	0.17	0.04	0.01							
16	<i>A. lifanensis</i>	0.14	0.14	0.05	0.14	0.14	0.16	0.08	0.08	0.08	0.07	0.06	0.19	0.06	0.06	0.06						
17	<i>A. loloensis</i>	0.16	0.13	0.06	0.14	0.14	0.15	0.07	0.05	0.08	0.06	0.04	0.17	0.04	0.01	0.00	0.06					
18	<i>A. mantzorum</i>	0.16	0.14	0.07	0.15	0.14	0.15	0.07	0.04	0.07	0.07	0.03	0.17	0.05	0.01	0.02	0.07	0.02				
19	<i>A. tuberodepressus</i>	0.16	0.15	0.07	0.15	0.14	0.15	0.08	0.06	0.08	0.07	0.04	0.18	0.05	0.02	0.02	0.07	0.02	0.02			
20	<i>A. cucae</i>	0.15	0.16	0.09	0.13	0.15	0.18	0.07	0.08	0.09	0.07	0.08	0.19	0.05	0.05	0.05	0.08	0.05	0.06	0.06		
21	<i>A. mengyangensis</i>	0.16	0.15	0.07	0.15	0.16	0.18	0.06	0.06	0.08	0.07	0.07	0.19	0.05	0.04	0.05	0.08	0.05	0.04	0.06	0.04	
22	<i>A. kangtingensis</i>	0.15	0.14	0.06	0.14	0.14	0.15	0.07	0.04	0.06	0.07	0.03	0.17	0.04	0.01	0.01	0.06	0.01	0.00	0.02	0.05	0.04

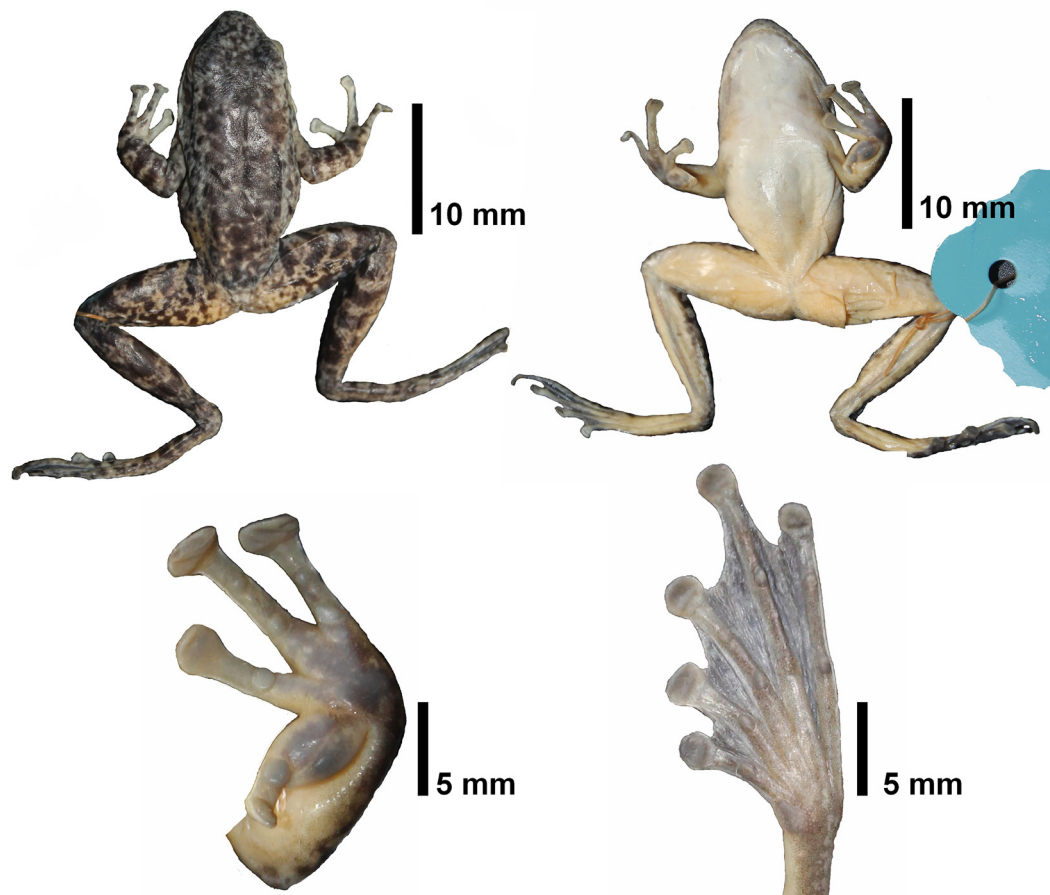


Figure 5 Male holotype in preservative A. Dorsal view B. Ventral view C. Ventral view of hand D. Ventral view of foot.

and tadpoles with gastromyzophorous) of the specimens to those of other species in the *A. marmoratus* group. Further, the new species is assigned to the genus *Amolops* due to the presence of ventral sucker in tadpole (tadpoles were identified with molecular method); a major diagnostic feature (Inger, 1966). *Amolops mahabharatensis* sp. nov. differed from other species of *Amolops* based on the following morphological characters: (1) male SVL (mean 37.1 mm, n=14), (2) dorsal surface granular, (3) head wider than longer, (4) distinct dorsolateral fold, (5) nuptial pad present on first finger of male (6) circummarginal grooves present on all digit discs, (7) toes fully webbed, (8) males with dual gular poaches, (9) vomerine teeth weakly developed, and (10) tadpoles with gastromyzophorous (ventral abdominal sucker) and distinct mouth parts (III:5+5/1+1:II).

Description of holotype (measurements provided in Table 5): Body size moderate (SVL 34.2 mm); head wider than long (HW 12.9, HL 9.7, HW:HL 133.5%) and flat; snout short and round in lateral view (SL:HL 50%); canthus rostralis distinct; nostril lateral; loreal region slightly concave, small eyes and convex (ED 2.8 mm); eye diameter shorter than snout length (ED:SL 58%); tympanum small (TYD:ED 46%); interorbital distance (IOD 4.4

mm), greater than width of the upper eyelid (UEW 3.4 mm), and internarial distance (ENL 1.9 mm); supratympanic fold present, extending from back of eye to shoulder; small tubercles present between the junction of supratympanic fold and upper mandible; vomerine teeth weakly developed; tongue lanceolate, deeply notched posteriorly; gular pouches present.

Arm robust (FAL=7.4 mm) and shorter than hand (HAL=10.6 mm); relative finger lengths I<II<IV<III; third finger (F3=6.7) shorter than arm (FAL=7.4); finger tips dilated with oval disks with circummarginal grooves; third disk width (FD3 1.5), greater than tympanum (TYD:FD3 84%); nuptial pad on first finger, without conical spines, fingers without distinct lateral fringes, webbing absent; rounded subarticular tubercles; formula for subarticular tubercles: 1, 1, 2, 1; outer metacarpal tubercle ovoid, flat and larger than inner tubercle, round and small.

Hindlimbs powerful and long, tibiotarsal articulation reaching the snout when hindlimb is kept parallel to the body; shank (SHL 23.2) longer than thigh (THL 20.7), tarsus (TL 10.6) and foot length (FOL 20.2); toes thin and long, relative lengths I<II<III<V<IV; toes tips with circular disks with circummarginal

grooves; diameter of fourth disks (TD 4 1.7); toes fully webbed; subarticular tubercles slightly ovoid, formula 1, 1, 2, 3, 2; inner metatarsal tubercle prominent and oval; outer metatarsal poorly developed; supernumerary and plantar tubercles absent.

Skin texture and colouration in life: Skin granular on the dorsal surface of head, body, limbs, fingers, toes and flank regions, intensity of granular warts increases towards vent; flanks with white larger granules; dorsolateral fold very weakly developed with warty granules; abdomen and ventral surfaces of head, body and thighs free of granules; tympanum smooth. Dorsum greyish olive; dorsolateral and supratympanic folds light brown; thighs with black bands; toe webbing brown; ventral region white; iris pale yellow to light gold, pupil black.

Colouration in preservation: Dorsum of head and body dark brown; flanks creamy; dorsal region of thigh brown, lateral surfaces of arms and thighs light rust; dorsal granules turned to black from creamy white; transverse cross-bars on dorsal surfaces of hands, shanks, tarsus and feet turned to grey; flank and ventral surfaces of hand and thigh region turned to creamy white; ventral surfaces of head and abdomen creamy white. Webbing of the toes turned to semi-transparent grey.

Variations: Morphometric variation of type specimens is presented in Table 4.

Sexual dimorphism: Some morphological characters showed sexual dimorphism. In SVL, females were significantly larger than males; males with sub-gular vocal sac (v.s. absence in females); distinct nuptial pads (v.s. absence in females); thick, robust forearms (v.s. thin and long in females).

Larvae: Two tadpoles of *Amolops mahabharatensis* sp. nov. (identity confirmed by molecular analysis) on Gosner stage 32 were examined (Figure 7). In dorsal view, body oval or elliptical, posterior region wider than the anterior, total body length (TL 34.8 mm), body length (BL 16.7 mm) 48% of TL and tail length (TAL 21.2 mm) 61% of TL. Head broad, snout rounded, eyes positioned dorsolaterally, naris oval and closer to the snout, ED (1.8 mm) 11% of BL, ENL (2.0 mm) 12% of BL, SN (1.8 mm) 11% of BL, SSD (8.1 mm) 48% of BL, IND (1.7 mm) 11% of BL and IOW (2.0 mm) 12% of BL. Open nostril; spiracle sinistral single, midventral, posterodorsally directed and extended as a short tube; spiracular opening oval; dextral vent tube opens at the margin of the ventral fin; intestinal coils invisible; tail musculature robust and greatly narrowing towards the tail tip; tail tip rounded; TMH (3.1 mm) 15% of TAL, UTF (0.82 mm) 4% of TAL, LTF (1.0 mm) 5% of TAL, TMW (2.5 mm) 12% of TAL, MTH (2.9 mm) 14% of TAL, dorsal fin originates behind body, upper tail fin originates at first quarter of tail body junction, lower tail fin origin near the middle of tail, upper tail fin higher than lower tail fin, both fins converged at the tip. In ventral view, a large sucker abdominal disk almost covers 80% of ventral body area. Oral disc: lips lacking papillae, three



Figure 6 Female-paratype in natural habitat.

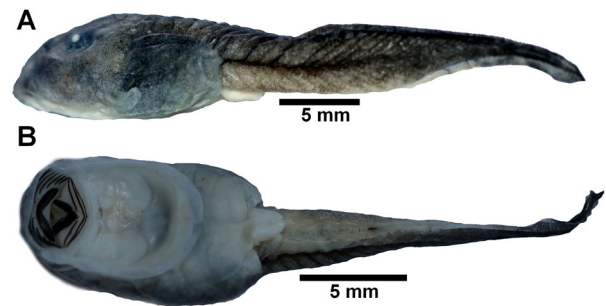


Figure 7 Tadpole of *Amolops mahabharatensis* sp. nov. A. Stage 32 larva in dorsolateral view B. Ventral view C. Mouth showing denticles

uninterrupted teeth row and five interrupted teeth row on the upper labium. The lower labium comprises of one interrupted teeth row followed by two uninterrupted teeth row. The teeth row: III:5+5/1+1:II. Beak completely black, margin serrate, about 64 serrates in upper beak and 36 in lower beak, upper beak inverted V-shaped, lower V-shaped.

Coloration of tadpoles in preservative: Dorsal and lateral surface of head, body and tail region dark brown, abdominal disc creamy white, abdomen light grey, ventral surface of tail creamy white.

Ecological notes: *Amolops mahabharatensis* sp. nov. inhabits torrent streams with high canopy cover. The vegetation is subtropical mixed forest. Adults of new species were collected from rocky boulders in a fast-flowing stream with more than 85% of rock cover (Figure 8). Tadpoles were attached on the rocks below water and were sampled with the help of a fishing net placed at the bottom of the rock. The tadpoles were rubbed and detached from rock by using a plastic slipper and collected into the net.

Table 4 Variable loadings for principal components with Eigen value greater than, from size corrected morphological traits.

Morphological traits	Male						Female					
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
Head length	0.086	-0.165	0.805	0.037	0.063	0.4	-0.321	0.424	0.747	-0.033	0.294	-0.163
Head width	0.166	0.159	0.007	-0.072	0.022	0.856	0.131	-0.407	0.817	-0.154	0.195	-0.032
snout length	0.431	0.581	0.47	-0.157	-0.197	0.107	0.702	0.083	0.165	0.676	-0.011	-0.076
Eye diameter	-0.263	0.042	-0.148	0.108	-0.821	-0.015	0.464	0.175	0.123	0.334	-0.256	0.6
Nostril to eye	0.262	0.298	0.601	0.044	-0.205	-0.071	-0.276	0.777	0.238	0.127	-0.408	-0.273
Width of upper eyelid	-0.015	0.452	-0.014	0.157	0.784	0.007	0.15	0.1	0.818	-0.031	-0.061	0.516
Inter-orbital width	0.213	0.773	0.042	0.095	0.1	0.131	0.044	-0.054	-0.022	0.995	-0.023	-0.053
Inter-Narial width	-0.536	-0.334	0.38	0.27	-0.187	0.449	0.511	-0.086	0.721	0.296	0.304	-0.163
Tympanum diameter	-0.045	0.422	0.671	0.138	0.217	0	0.334	-0.541	-0.105	-0.162	0.367	-0.65
Length of arm	-0.029	-0.034	-0.716	0.132	-0.426	0.382	-0.12	-0.018	0.17	-0.138	0.954	-0.127
Length of hand	-0.23	-0.81	-0.332	0.139	-0.154	0.22	-0.622	0.45	0.323	-0.266	0.157	0.449
Length of 1st finger	0.528	0.675	0.065	0.104	0.176	-0.206	-0.053	-0.005	0.025	0.923	-0.225	0.299
Length of 2nd finger	0.495	0.682	0.192	0.216	0.204	0.021	0.023	-0.184	0.271	0.642	0.341	0.583
Length of 3rd finger	0.48	0.725	0.142	0.009	0.156	0.146	0.943	0.002	0.263	0.079	0.012	0.07
Length of 4th finger	0.329	0.783	-0.111	0.093	-0.094	0.179	0.757	-0.53	-0.241	0.256	0.001	0.138
Length of femur	0.465	0.098	0.083	0.764	0.138	0.318	-0.746	0.015	-0.205	0.219	0.593	0.047
Length of tibia	0.284	0.214	0.193	0.857	-0.001	0.004	-0.943	0.016	-0.219	0.112	0.163	-0.125
Length of tarsus	0.212	-0.172	-0.205	0.804	-0.113	-0.285	-0.597	0.285	-0.275	-0.311	0.434	-0.449
Length of meta-tarsus	0.646	0.422	0.018	0.448	0.052	0.14	-0.737	0.049	0.588	0.056	-0.191	0.166
Length of 1st toe	0.855	0.254	0.057	0.245	0.057	-0.025	-0.083	0.972	-0.003	0.009	0.061	0.183
Length of 2nd toe	0.865	0.207	0.006	0.314	0.125	-0.108	-0.126	0.95	-0.142	-0.208	0.087	0.104
Length of 3rd toe	0.917	0.276	0.055	0.167	0.036	0.073	0.505	0.819	0.192	0.034	-0.03	0.006
Length of 4th toe	0.866	0.371	0.171	0.135	0.055	0.153	0.361	0.204	0.731	0.301	-0.342	0.229
Length of 5th toe	0.835	0.259	0.164	0.15	0.07	0.185	0.145	0.466	0.704	0.212	-0.234	0.366
Cumulative % of Variance Explained	43.015	55.415	64.782	72.149	77.733	83.23	31.038	56.374	70.269	83.308	91.803	97.368



Figure 8 A. Habitat and B. Microhabitat of *Amolops mahabharatensis* sp. nov. at Siraichuli Hill, Chitwan district, Nepal.

Comparisons

Based on the phylogenetic analysis, *Amolops mahabharatensis* sp. nov. is the member of *A. marmoratus* species group (*A. afghanus*, *A. indoburmensis*, *A. marmoratus*, *A. nepalicus* and *A. panhai*) and is morphologically distinct from the currently known members of *A. marmoratus* group and all other congeneric species (Table 6).

Amolops mahabharatensis sp. nov. differs from *A. afghanus* by having granular dorsal surface (vs. less granular), smaller body size both in males SVL: 33.9–39.1 (vs. large size, SVL: 45.6–62.9) and females SVL: 61.0–71.7 (vs. large size, SVL: 67.7–94.1) and relatively larger tympanum (about 8.2% of SVL) (vs. smaller diameter about 5% of SVL) (Dever et al. 2012).

Amolops mahabharatensis sp. nov. differs from *A. indoburmanensis* by having small body size both in males (vs. large size, SVL: 59.0–86.0) and females (vs. large size, SVL: 63.0–106.0) (, head wider than long (vs. longer than wide), finely granulated (vs. coarsely granulated in dorsum body and limbs) (Dever et al. 2012).

Amolops mahabharatensis sp. nov. differs from *A. marmoratus* by having head wider than long (vs. longer than wide), distinct tympanum (vs. indistinct), supernumerary tubercles on finger absent (vs. supernumerary tubercles on three outer fingers).

Amolops mahabharatensis sp. nov. differs from *A. nepalicus* by having head wider than long (vs. longer than wide), keratodont formula of tadpole – III:5-5/1-1:II (vs. II:4-4/1-1:II).

Amolops mahabharatensis sp. nov. differs from *A. panhai* by having large body size in female, SVL: 61.0–71.7 (vs. small size,

SVL: 47.2–57.5), head wider than long (vs. longer than wide), gular pouches present in males (vs. absent), keratodont formula of tadpole II:4-4/1-1:II (vs. III:5-5/1-1:II).

Amolops mahabharatensis sp. nov. further differs from *A. formosus*, *A. monticola* and *A. himalayanus* by having granular dorsal surface (vs. smooth), circummarginal groove on first finger present (vs. absent), dorsal body greyish olive (vs. dorsum dark green) and distinctive dorsolateral fold absent (vs. present in *A. monticola*).

Amolops mahabharatensis sp. nov. further differs from all other species of *Amolops* based on the following characters: gular pouches present in males (vs. absent in *A. albispinus*, *A. bellulus*, *A. caelumnoctis*, *A. chakrataensis*, *A. cremnobatus*, *A. daiyunensis*, *A. formosus*, *A. gerbillus*, *A. granulosus*, *A. hainanensis*, *A. jinjiangensis*, *A. kaulbacki*, *A. larutensis*, *A. lifanensis*, *A. loloensis*, *A. mantzorum*, *A. minutus*, *A. nidorbellus*, *A. nyingchiensis*, *A. ricketti*, *A. shuichengicus*, *A. sinensis*, *A. tuberodepressus*, *A. viridimaculatus*, *A. xinduoqiao* and *A. yatseni*), head wider than long (vs. longer than wide or head in *A. akhaorum*, *A. albispinus*, *A. assamensis*, *A. australis*, *A. bellulus*, *A. caelumnoctis*, *A. chunganensis*, *A. compotrix*, *A. cucae*, *A. daorum*, *A. gerbillus*, *A. gerutu*, *A. granulosus*, *A. hongkongensis*, *A. indoburmanensis*, *A. iriodes*, *A. jaunsari*, *A. jinjiangensis*, *A. kaulbacki*, *A. kohimaensis*, *A. larutensis*, *A. lifanensis*, *A. loloensis*, *A. marmoratus*, *A. mengdingensis*, *A. mengyangensis*, *A. minutus*, *A. monticola*, *A. nepalicus*, *A. nyingchiensis*, *A. panhai*, *A. shuichengicus*, *A. splendissimus*, *A. torrentis*, *A. tuberodepressus*, *A. viridimaculatus*, *A. vitreus*, *A. wenshanensis*, *A. xinduoqiao* and *A. yunkaiensis*), distinct tympanum (vs. indistinct *A. albispinus*, *A. gerbillus*, *A.*

Table 5 Measurements (in mm; mean±SD) of the type series of *Amolops mahabharatensis* sp. nov. See Material and methods for abbreviations.

	CDZMTU0110	CDZMTU0111	CDZMTU0112	CDZMTU0113		CDZMTU0114	CDZMTU0115	
	Holotype	Paratype	Paratype	Paratype	Mean ± SD	Paratype	Paratype	Mean ± SD
	Male	Male	Male	Male		Female	Female	
SVL	34.2	33.91	39.11	38.66	36.5 ± 2.8	71.79	65	68.4 ± 4.8
HL	9.73	10.42	13.54	12.36	11.5 ± 1.8	20.16	20.78	20.5 ± 0.4
HW	12.99	11.73	12.46	12.71	12.5 ± 0.5	22.69	20.1	21.4 ± 1.8
SL	4.94	4.07	5.41	5.82	5.1 ± 0.8	5.3	4.99	5.1 ± 0.2
ED	2.89	3.94	3.43	4.04	3.6 ± 0.5	8.91	8.69	8.8 ± 0.2
ENL	1.93	3.34	4.02	4.46	3.4 ± 1.1	4.55	4.94	4.7 ± 0.3
UEW	3.41	3.64	4.14	2.84	3.5 ± 0.5	3.55	3.96	3.8 ± 0.3
IOW	4.45	5.09	4.12	5.72	4.8 ± 0.7	6.9	5.76	6.3 ± 0.8
IND	2.39	3.52	3.25	3.9	3.3 ± 0.6	6.14	6.03	6.1 ± 0.1
TYD	2.33	2.31	1.79	3.16	2.4 ± 0.6	2.76	3.32	3 ± 0.4
FAL	7.44	7.92	6.13	7.7	7.3 ± 0.8	11.97	10.55	11.3 ± 1
HAL	10.67	8.72	10.91	13.57	11 ± 2	33.11	28.69	30.9 ± 3.1
F1	4.69	4.73	5.17	3.13	4.4 ± 0.9	5.19	4.02	4.6 ± 0.8
F2	5.57	6.79	4.88	5.9	5.8 ± 0.8	5.09	2.04	3.6 ± 2.2
F3	6.76	8.27	7.75	9.13	8 ± 1	6.63	6.73	6.7 ± 0.1
F4	5.22	6.99	4.84	5.08	5.5 ± 1	7.61	5.77	6.7 ± 1.3
THL	20.77	19.36	19.05	23.85	20.8 ± 2.2	38.89	33.59	36.2 ± 3.7
SHL	23.26	20.75	20.88	25.64	22.6 ± 2.3	42.97	39.81	41.4 ± 2.2
TL	10.61	8.95	9.82	11.47	10.2 ± 1.1	23.09	21.07	22.1 ± 1.4
FOL	20.25	18.29	19.32	23.48	20.3 ± 2.2	32.42	31.5	32 ± 0.7
T1	5.06	3.44	5.12	6.86	5.1 ± 1.4	5.66	4.52	5.1 ± 0.8
T2	7.54	4.16	7.58	6.23	6.4 ± 1.6	7.61	6.68	7.1 ± 0.7
T3	9.23	6.46	10.55	10.21	9.1 ± 1.9	7.36	5.86	6.6 ± 1.1
T4	13.95	11.79	14.12	15.71	13.9 ± 1.6	7.38	6.43	6.9 ± 0.7
T5	9.44	7.98	7.61	10.48	8.9 ± 1.3	6.71	5.63	6.2 ± 0.8

granulosus, *A. jinjiangensis*, *A. lifanensis*, *A. loloensis*, *A. marmoratus*, *A. shuichengicus*, *A. viridimaculatus* and *A. xinduoqiao*), presence of vomerine teeth (vs. absent in *A. daiyunensis*, *A. daorum*, *A. hainanensis*, *A. hongkongensis*, *A. torrentis*, and *A. wuyiensis*), absent of distinctive dorsolateral fold (vs. present *A. akhaorum*, *A. aniqiaoensis*, *A. archotaphus*, *A. bellulus*, *A. chakrataensis*, *A. chayuenensis*, *A. chunganensis*, *A. compotrix*, *A. cremnobatus*, *A. cucae*, *A. daorum*, *A. gerbillus*, *A. iriodes*, *A. jaunsari*, *A. jinjiangensis*, *A. kohimaensis*, *A. mengdingensis*, *A. mengyangensis*, *A. minutus*, *A. nyingchiensis*, *A. shuichengicus*, *A. vitreus*, *A. wenshanensis* and *A. xinduoqiao*).

Supplementary description of *Amolops nepalicus* (Yang, 1991), based on specimens from Dobhan population (DP)

Diagnostic keys to the *Amolops nepalicus* (Yang, 1991)

1. Head slightly longer than wide
2. Tympanum visible
3. First and second fingers equal in length
4. Supernumerary tubercles at bases of all fingers
5. Toes fully webbed; subarticular tubercles small; inner metatarsal tubercle present
6. No dorsolateral fold; an obvious temporal fold

Comparisons

Based on the diagnostic characters of the *Amolops nepalicus* (Yang, 1991), DP is morphologically similar with *A. nepalicus* and is morphologically distinct from the currently known members of *A. marmoratus* group (*A. afghanus*, *A. indoburmensis*,

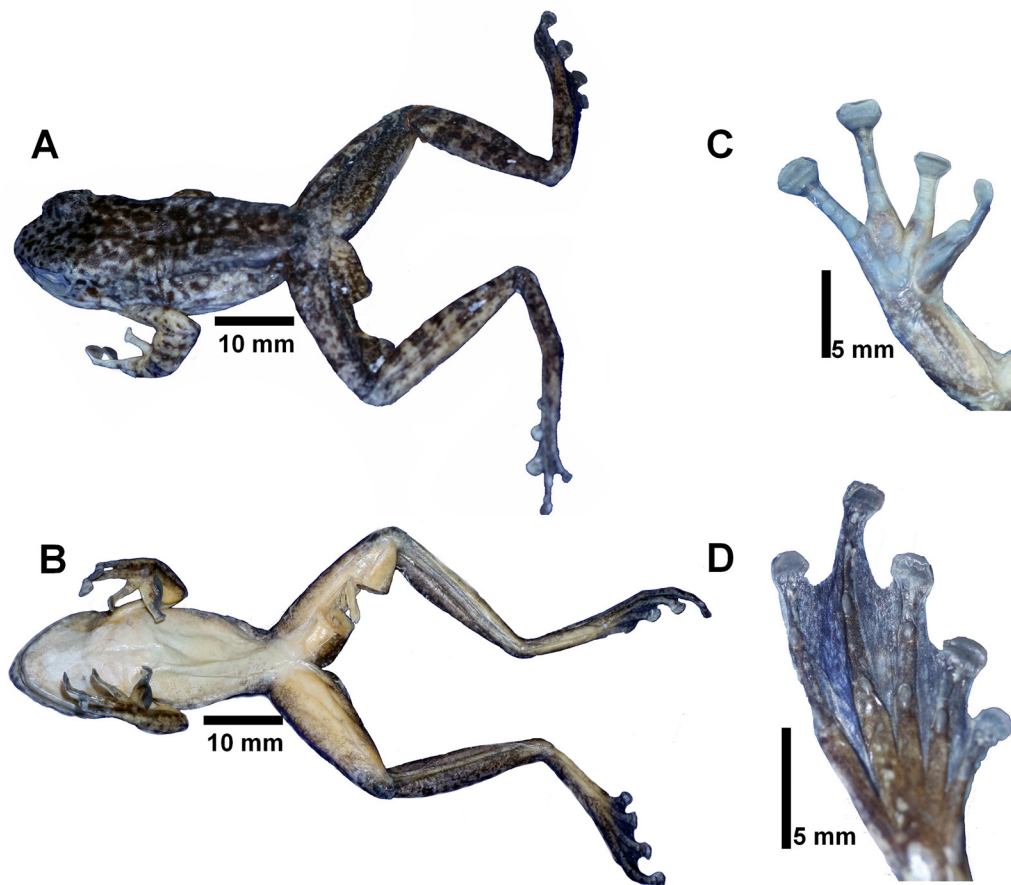


Figure 9 *Amolops nepalicus* in preservative A. Dorsal view B. Ventral view C. Ventral view of hand D. Ventral view of foot.

A. marmoratus, *A. mahabharatensis* sp. nov. and *A. panhai*) and all other congeneric species (Table 6).

Amolops nepalicus differs from *A. afghanus* by having small body size, SVL: smaller body size both in males SVL: 33.9–39.1 (vs. large size, SVL: 45.6–62.9) and females SVL: 61.0–71.7 (vs. large size, SVL: 67.7–94.1), head longer than wide (vs. wider than long), outer metatarsal tubercle (vs. absent) (Dever et al. 2012). *Amolops nepalicus* differs from *A. indoburmanensis* by having small body size (vs. large size), head longer than wide (vs. wider than long), outer metatarsal tubercle (vs. absent) (Dever et al. 2012) (Table 6). *Amolops nepalicus* differs from *A. marmoratus* by having head wider than long (vs. longer than wide), distinct tympanum (vs. indistinct). *Amolops nepalicus* differs from *A. mahabharatensis* sp. nov. by having longer head than wide (vs. wider than long), , keratodont formula of tadpole: II:4-4/1-1:II (vs. III:5-5/1-1:II). *Amolops nepalicus* differs from *A. panhai* by having outer metatarsal tubercle (vs. absent), keratodont formula of tadpole: II:4-4/1-1:II (vs. III:4-4/1-1:II). *Amolops nepalicus* further differs from *A. formosus*, *A. monticola* and *A. himalayanus* by having granular dorsal surface (vs. smooth), circummarginal groove on first finger present (vs. absent), and distinctive

dorsolateral fold absent (vs. present in *A. monticola*).

Further, *A. nepalicus* is differ from several name bearing *A. marmoratus* complex group such as *Rana latopalmata* by having smaller body size (vs. larger SVL; male: 77–90 mm and female: 90–100 mm), keratodont formula of tadpole: II:4-4/1-1:II (vs. III:5-5/1-1:II) (Boulenger 1920) and with *Philautus argus* by having larger body size (vs. smaller SVL; male: 27 mm), head longer than wide (vs. wider than long) (Boulenger 1920).

Holotype: Adult male (UMMZ 132063), from “Sabha Khola, Sankhuwasabha district, eastern Nepal”, collected by Douglas Lay on 02 June 1973.

Examined specimens: Specimens of *A. nepalicus* were collected from fast flowing stream of Dobhan, Sankhuwasabha district, Nepal, 27.55432 °N, 87.299366 °E; elevation 848 m a.s.l. collected by Janak Raj Khatiwada between 19:00 and 22:00h on 25th May 2015 and deposited in the collection of Central Department of Zoology, Tribuvan University, Kirtipur, Kathmandu, Nepal. Description: The given description is based on the collection of specimens from Dobhan, Sankhuwasabha district, eastern Nepal (Figure 9 and Table 7).

Mean body size (male: mean SVL = 41.1 mm, $n = 7$ and

Table 6 Morphological comparisons between *Amolops mahabharatensis* sp. nov. with other members of genus *Amolops*. Morphological abbreviations are given in the Table 1. “–” denotes the information missing.

	Body size (SVL)		Head	Externally visible vocal sac in males	Vomerine teeth	Dorsolateral fold	Outer metatarsal tubercle	Dorsal body colouration (in life)	Keratodont row formulae in tadpole	Distribution	Reference
	Male	Female	HL>HW								
<i>mahabharatensis</i> sp. nov.	33.9–39.1	61.0–71.7	HL<HW	present	distinct	absent	present	Dorsum greyish olive	III:5+5/1+1:II	Mahabharat range of Nepal	This study
<i>afghanus</i>	45.6–62.9	67.7–94.1	HL<HW	present	distinct	absent	absent	Above brown, greyish or grey, with darker spots	–	Nampoung valley, Myanmar	Günther (1858)
<i>akhaorum</i>	34.9–37.2	58.8–62.5	HL>HW	present	distinct	present	absent	Dorsum dark green, with raised black tubercles	–	Nam Ha National Protected Area, Vieng Phou Kha District, Namtha Province, Laos	Stuart <i>et al.</i> (2010)
<i>albispinus</i>	36.7–42.4	43.1–51.9	HL=HW	absent	indistinct	absent	absent	Dorsal surface olive-brown with raised dark brown blotches	–	Mt. Wutong, Shenzhen City, Guangdong Province, China	Sung <i>et al.</i> (2016)
<i>aniquiaoensis</i>	–	52.0	–	present	indistinct	present	absent	Light green	–	Aniqiao and Medog, Xizang, China	Zhao <i>et al.</i> (2005)
<i>archotophus</i>	38.2–42.1	58.8–62.5	–	present	distinct	present	present	Olive green	–	Chiang Mai Province, northwestern Thailand	Inger and Chanard (1997)
<i>assamensis</i>	52.8–61.5	82.50–94.4	HL>HW	present	distinct	absent	present	Olive green with rounded or irregular brown patches	III:5+5/1+1:II	Assam, north-eastern India	Sengupta <i>et al.</i> (2008)
<i>australis</i>	28.7–32.7	45.8–47.0	HL>HW	present	distinct	absent	present	Light gray with irregular dark-brown spots	–	Johor, Peninsular Malaysia,	Chan <i>et al.</i> (2018)
<i>bellulus</i>	45.9–50.1	63.6	HL>HW	absent	distinct	present	absent	Olive green with irregular brown spots	III: 4+4/1+1:II	Yunnan, China, border between Yunnan and Myanmar	Liu <i>et al.</i> (2000)
<i>caelumnocis</i>	71.3–73.7	78–90.6	HL>HW	absent	distinct	absent	present	Dark purple with round light-yellow spots	–	Mount Huanglanshan, Yunnan, China	and Fei <i>et al.</i> (2010) Rao and Wilkinson (2007)
<i>chakrataensis</i>	–	55.0	HL<HW	–	distinct	present	absent	Brown	–	Chakrata, Dehra Dun, Uttarakhand, India	Ray (1992)
<i>chayuensis</i>	–	51.4	–	present	indistinct	present	absent	Slaty brown	–	Chayu, Xizang, China	Sun <i>et al.</i> (2013)
<i>chunganensis</i>	34.0–39.0	44.0–54.0	HL>HW	present	distinct	present	absent	Green	–	Fujian, China	Pope (1929)
<i>compotrix</i>	31.4–42.6	55.6–56.9	HL>HW	present	distinct	present	absent	Reddish brown above but light beneath	–	Southern highlands of Laos and the adjacent central highlands of Vietnam	Bain <i>et al.</i> (2006)
<i>cremnobatus</i>	32.0–34.0	–	HL<HW	absent	distinct	present	–	Yellowish brown	–	Nam Phao River, Khammouan, Laos	Inger and Kottelat (1998)
<i>cucae</i>	40.7–44.6	65.8–68.0	HL>HW	present	distinct	present	present	Light green	–	Lao Cai Province, Vietnam	Bain <i>et al.</i> (2006)
<i>daiyunensis</i>	36.0–58.0	44.0–63.0	HL<HW	absent	indistinct	absent	absent	Yellowish brown	–	Dehua, Daiyun Shan, Fujian, China	Liu and Hu (1975)

(Continued Table 6)

	Body size (SVL)		Head	Externally visible vocal sac in males	Vomerine teeth	Dorsolateral fold	Outer metatarsal tubercle	Dorsal body colouration (in life)	Keratodont row formulae in tadpole	Distribution	Reference
	Male	Female									
<i>daorum</i>	32–38	53–58	HL>HW	present	absent	present	absent	Green with black spots	–	Sa Pa village, near O Qui Ho Pass, Lao Cai Province, Vietnam	Bain <i>et al.</i> (2003)
<i>formosus</i>	–	63.1	HL<HW	–	Present	absent	present	Dark green with irregular black spots	–	North eastern India and eastern Nepal	Gunther (1875) and this study
<i>gerbillus</i>	–	–	HL>HW	–	indistinct	present	present	Dark gray	–	Tibet, China, northern and north-eastern India and adjacent Myanmar	Annandale (1912)
<i>gerutu</i>	34.0–37.2	45.0–58.2	HL>HW	present	Present	absent	present	Brown with irregular light-gray patches	–	Peninsular Malaysia	Chan <i>et al.</i> (2018)
<i>hainanensis</i>	71.2–93.1	67.8–78.2	–	absent	–	absent	absent	Olive with black and pale olive markings	III:2-2/1-1:II	Hainan, China	Boulenger (1900)
<i>himalayanus</i>	77.0–90.0	90.0–100.0	HL<HW	absent	present	absent	present	Olive or greyish above	III:4-4/1-1:II	Bhutan, north-eastern India	Boulenger (1888), (Boulenger 1920), Nidup <i>et al.</i> (2016)
<i>hongkongensis</i>	34.0–41.0	31.0–48.0	HL=HW	present	distinct	absent	present	Yellowish brown	III:1-1/1-1:II	Guangdong and Hong Kong, China	Pope and Romer (1951) and Fei <i>et al.</i> (2010)
<i>indoburmanensis</i>	59.0–86.0	63.0–106.0	HL>HW	present	distinct	absent	present	Brown with darker brown spots	–	Chin and Rakhine State, Myanmar	Dever <i>et al.</i> (2012)
<i>iriodes</i>	39.0–43.0	63.0	HL>HW	present	distinct	present	present	Iridescent light green with some black spots	–	Mt. Tay Con Linh II, Ha Giang Province, north-eastern Vietnam	Bain and Truong (2004)
<i>jaunsari</i>	–	–	HL=HW	present	distinct	present	present	Dark olive green	–	Sahiya, Dehradun District, Uttarakhand, India	Ray (1999)
<i>jinijiangensis</i>	44.0–54.0	58.0–65.0	HL>HW	absent	indistinct	present	–	Green with brown spots	III:3-3(4-3)/1-1:II	Yajiang, Deqing and Zhongdian, Yunnan, and Daliangshan, Sichuan, China	Su <i>et al.</i> (1986) and Fei <i>et al.</i> (2010)
<i>kaulbacki</i>	70.0–72.0	63.0	HL=HW	absent	distinct	absent	present	Bluish or green	–	North-eastern India, and northern Myanmar	Smith (1940)
<i>kohimaensis</i>	42.8–48.6	–	HL>HW	present	distinct	present	present	Medium brown	–	Jotsoma village, Kohima district, Nagaland, northeast India	Biju <i>et al.</i> (2010)
<i>larutensis</i>	35.0–40.0	53.0–57.0	HL>HW	absent	distinct	absent	present	Darker olive with irregular darker and lighter spots	–	Perak, Malaysia	Boulenger (1899)
<i>lijianensis</i>	52.0–56.0	61.0–79.0	HL=HW	absent	indistinct	absent	present	Olive on back, with dark irregular marks	III:4-4/1-1:II	Lishan, Maoxian and Wenchuan, Sichuan Province, China	Liu (1945)
<i>loloensis</i>	54.5–62.0	69.5–77.5	HL=HW	absent	indistinct	absent	absent	Dark green with many reddish large brown spots	III:4-4/1-1:II	Yunnan and Sichuan Provinces, China	Liu (1950)
<i>marmoratus</i>	38.2–48.1	69.8–79.0	HL>HW	present	indistinct	absent	present	–	–	Myanmar, India, Bhutan, Nepal and China	Dever <i>et al.</i> (2012)
<i>mantzorum</i>	48.8–57.6	57.5–72.0	HL<HW	absent	distinct	absent	absent	Brown back with a few green blotches	III:4-4/1-1:II	Yunnan and Sichuan Provinces, China	Fei <i>et al.</i> (2010)
<i>medogensis</i>	95.0	92.6	–	present	distinct	absent	–	Green with brown blotches	–	Medog, Tibet, China	Zhao <i>et al.</i> (2005)

(Continued Table 6)

	Body size (SVL)		Head	Externally visible vocal sac in males	Vomerine teeth	Dorsolateral fold	Outer metatarsal tubercle	Dorsal body colouration (in life)	Keratodont row formulae in tadpole	Distribution	Reference
	Male	Female									
<i>mengdingensis</i>	37.1–40.2	64.3	HL>HW	present	present	present	present	Light green with some dark spots	–	Southwestern Yunnan, China	Yu <i>et al.</i> (2019)
<i>mengyangensis</i>	38.3–38.7	60.0	HL>HW	present	absent	present	–	Olive brown	–	Xishuang Banna, southern Yunnan Province, China	Wu and Tian (1995)
<i>minutus</i>	29.7–36.4	38.5–50.2	HL>HW	–	present	present	absent	Marble brown with green speckles	–	Lai Chau Province, Vietnam	Orlov and Ho (2007)
<i>monticola</i>	41.0	65.0–75.0	HL=HW	present	present	absent	absent	Green	–	North-eastern India to eastern Nepal	Anderson (1871)
<i>nepalicus</i>	35.6–46.6	45.3–79.0	HL>HW	present	present	absent	present	Light brown or white with dark brown spots	II:4-4/1-1:II	Sabha Khola and Dobhan, Sankhuwasabha district, Nepal	Yang (1991) and this study
<i>nidorhellus</i>	76.4–82.3	85.4–98.0	HL<HW	absent	present	absent	present	Brown with distinct green blotches	–	Kohima district, Nagaland, India	Biju <i>et al.</i> (2010)
<i>nyingchiensis</i>	48.5–58.3	57.6–70.7	HL>HW	absent	present	present	absent	Light brown or yellowish brown	–	Tibet, China	Jiang <i>et al.</i> (2016)
<i>panhai</i>	31.7–33.9	47.2–57.5	HL>HW	present	present	absent	absent	–	II:4-4/1-1:II	Central and western Thailand and Taninthary, Myanmar	Matsui and Nabhitabhata (2006)
<i>ricketti</i>	42.0–60.5	53.5–67.0	HL<HW	absent	present	absent	absent	Olive brown with irregular dark spots	III:1-1/1-1:II	China and Vietnam	Fei <i>et al.</i> (2010)
<i>shuichengicus</i>	34.6–39.6	48.5–55.5	HL>HW	absent	present	present	absent	Brown with light green spots	–	Guizhou, China	Lyu <i>et al.</i> (2019b)
<i>sinensis</i>	40.2–46.5	47.7–52.7	HL<HW	absent	present	absent	absent	Dark green	–	Guangdong to northeastern Guangxi and southwestern Hunan, China	Lyu <i>et al.</i> (2019a)
<i>spinapectoralis</i>	41.0–53.2	52.3–66.9	HL<HW	present	present	absent	absent	Olive brown	–	Northern and central montane Vietnam	Inger <i>et al.</i> (1999)
<i>splendissimus</i>	62.6–75.6	69.3–96.8	HL=HW	–	present	absent	present	Dark purple with yellow spots	–	Duong district, Lai Chau Province, Vietnam and Yunnan, China	Orlov and Ho (2007)
<i>torrentis</i>	27.8–32.8	34.4–41.4	HL=HW	present	absent	absent	present	Brownish black with irregular dark brown spots	III:2+2/1+1:II	Hainan Island, and Guangdong, China	Smith (1923)
<i>tubero depressus</i>	44.3–57.6	60.8–71.1	HL>HW	absent	present	absent	absent	Brown with irregular green or bluish green spots	–	Yunnan, China	Liu and Yang (2000)
<i>viridimaculatus</i>	77.0	90.0	HL>HW	absent	present	absent	present	Brown with distinct green spots	III:4+4/1+1:II	Yunnan, China and northern Vietnam	Jiang (1983) and Fei <i>et al.</i> (2010)
<i>vitreus</i>	37.5–43.6	–	HL>HW	present	present	present	present	Green with dark brown dots	–	Phongsaly Province, Laos, and Dien Bien Province, Vietnam	Bain <i>et al.</i> (2006)
<i>wenshanensis</i>	35.7–39.9	43.7–45.6	HL>HW	present	present	present	absent	Green	–	Wenshan city, Yunnan and Jingxi County, Guangxi, China	Yuan <i>et al.</i> (2018)
<i>wuyiensis</i>	–	–	HL<HW	present	present	absent	–	Olive brown	I:1+1/1+1:II	Zhejiang, northern Fujian and eastern Anhui, China	Fei <i>et al.</i> (2010)
<i>xinduiqiao</i>	41.1–47.5	49.5–56.6	HL=HW	absent	present	present	absent	Gray brown	–	Xinduqiao, Kangding, Sichuan, China	Fei <i>et al.</i> (2017)
<i>yatseni</i>	39.3–44.7	42.1–48.9	HL<HW	absent	present	absent	absent	Gray brown	–	Guangdong, China	Lyu <i>et al.</i> (2019a)
<i>yunkaensis</i>	31.8–34.1	35.2–39.0	HL>HW	present	absent	absent	absent	Olive-brown or light brown	–	Guangdong, China	Lyu <i>et al.</i> (2018)

female: mean SVL = 60.0 mm, Table 7); head relatively wide (HW 33% of SVL and HL 30% of SVL); canthus rostralis distinct; eye moderate (37% of HW); pupil round; tympanum small and round (37% of ED); inter-orbital distance (27% of HW) is greater than the inter-narial distance (34% of HW); snout flat and round (22% of HW) and is shorter than nostril to eye distance (18% of HW); width of upper eyelid (25% of HW) almost equal in length as the interorbital width (27% of HW); vomerine teeth present between choanae; dorsolateral folds indistinct. Arm shorter (LA 21% of SVL) than length of hand (LH 47% of SVL); fingers slender, free of webbing and tips with large and wide disc with circummarginal groove which are larger than those of the toes; relative length of fingers from shortest to longest $F1 \leq F2 < F4 < F3$; subarticular tubercles distinct and bulging; supernumerary tubercles small and round, located at bases of all fingers. Hindlimbs moderately slender; length of femur 51% of SVL; length of tibia 56% of SVL; length of tarsus 31% of SVL, length of meta-tarsus 45% of SVL. Tibiotarsal articulation reaches the snout when the hindlimb is kept parallel to the body. Toes strong and long with large disc in third and fourth digits; relative length of toes $T1 < T2 < T5 < T3 < T4$; toes fully webbed; subarticular tubercles small, round and distinct; metatarsal tubercle indistinct. Skin with few rounded tubercles scattered on back; dorsolateral fold indistinct; small tubercles on outer side of thigh; belly smooth.

Coloration (in alcohol): Light brown or white on back with dark brown spots on tubercles; darker brown and black crossbars on limbs; belly yellowish grey. Male with paired gular pouches.

Distribution: This species is known with certainty only from the Sabha Khola and Dobhan, Sankhuwasabha district, Nepal.

Ecological notes: The species inhabits torrent habitats with high canopy cover. The vegetation is subtropical mixed forest. Adults of *A. nepalicus* were collected from rocky surfaces in the fast-flowing stream with more than 90% of rock cover.

Identification *Amolops* populations from Kimathanka and Lamatar as *Amolops formosus* (Guenther, 1875)

Diagnostic keys to the *Amolops formosus* [based on the original description detailed by Guenther (1875) and measurements of the holotype (BMNH 1947.2.4.18)].

1. Snout flat, short, rounded, with distinct canthus rostralis
2. Tympanum very small, only as large as the disk of the third finger
3. Toes fully webbed, metatarsal tubercle, indistinct
4. Skin quite smooth
5. Vomerine teeth presence between choanae
6. Dorsolateral folds indistinct
7. Dorsum green, marbled with black, the black spots enclosing a number of small whitish dots.

Comparisons

Based on the morphological characters of the *Amolops formosus* (holotype BMNH 1947.2.4.18)], KLP is morphologically similar with *A. formosus* and is morphologically distinct from all other congeneric species (Table 6). *A. formosus* differs from *A. himalayanus* by having distinct tympanum (vs. indistinct), circummarginal groove on first finger present (vs. absent), tibiotarsal articulation reaches to the snout (vs. reaches beyond the tip of the snout) and distinctive dorsolateral fold absent (vs. present in *A. monticola*).

Holotype: Adult male (BMNH 1947.2.4.18, formerly 1874.4.17.281), from "Khasi Hills, Assam, India", collected by Thomas Caverhill Jerdon.

Examined specimens: Specimens of *A. formosus* were collected from fast flowing mountainous stream of Kimathanka, Sankhuwasabha district (27.839452° N, 87.393538° E; elevation 2508 m a.s.l.) and Lamatar, Taplejung district, Nepal (27.5361° N, 87.80802° E; elevation 1586 m a.s.l.) collected by Janak Raj Khatiwada between 19:00 and 22:00 h on 24th June 2015 and deposited in the collection of Central Department of Zoology, Tribuvan University, Kirtipur, Kathmandu, Nepal.

Description: The given description is based on the collection of specimens from Sankhuwasabha and Taplejung district, eastern Nepal (Figure 10 and Table. 8).

Female body size (SVL = 63.1–73.1 mm, $n = 3$); head relatively wider (HW 27% of SVL and HL 17% of SVL); canthus rostralis distinct; eye of moderate (39% of HW); pupil round; tympanum small and round (3% of SVL); inter-orbital distance (8% of SVL) greater than the inter-narial distance (9% of SVL); snout flat and rounded, snout (8% of SVL) longer than nostril to eye distance (8% of SVL); width of upper eyelid (4% of SVL) almost half length to the interorbital width; presence of small granules at angle of jaws and extra tympanic area; dorsolateral folds indistinct. Arm shorter (12% of SVL) than LH (length of hand 48% of SVL); fingers muscular, free of webbing, finger tips with large and wide disc with circummarginal groove and larger than those of the toes; relative length of fingers from shortest to longest $F1 < F2 < F4 < F3$; subarticular tubercles distinct and oval; elongated inner metacarpal tubercle, smaller than outer metacarpal. Tibiotarsal articulation reaches the snout when the hindlimb is kept parallel to the body. Hindlimbs muscular and slender; length of femur 48% of SVL; length of tibia 53% of SVL; length of tarsus 37% of SVL, length of meta-tarsus 21% of SVL. Toes strong and long with large disc in third and fourth digits; relative length of toes $T1 < T2 < T5 < T3 < T4$; toes fully webbed, subarticular tubercles oval and distinct; metatarsal tubercle indistinct.

Colouration: In life: skin smooth, dorsum green with irregular distinct chocolate coloured blotches with yellow dots. Ventral light and abdomen greenish. In alcohol: green colour faded to grey; the chocolate coloured blotches changed to

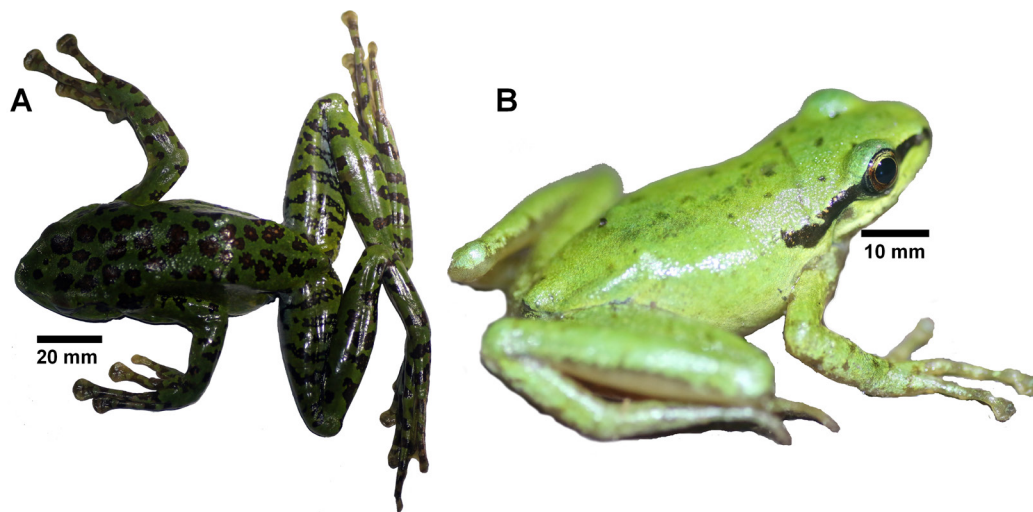


Figure 10 *Amolops formosus* A. Adult female B. Juvenile

brown.

Distribution: This species is distributed in Assam and Sikkim, India and eastern Nepal (Günther, 1875; Schleich and Kästle, 2002; Dinesh *et al.*, 2009) and in this study recorded above 1500 m in the eastern Nepal.

4. Discussion

In this study we have demonstrated significant differences in molecular and morphology between three species of *Amolops* currently distributed in the Mahabharat range of Nepal and with other closely related taxa. The population of *Amolops* from middle mountain range of Mahabharat range of Nepal are described as a new species (*Amolops mahabharatensis* sp. nov.). Previously, the *Amolops* population of the middle mountainous range of Nepal were identified as *Amolops marmoratus* (Dubois 2000; 2004; Nanho & Ouboter 1987; Schleich & Kästle 2002; Shah & Tiwari 2004). However, our results show the presence of three species in the middle mountainous regions of Nepal. Although, *Amolops mahabharatensis* sp. nov. resembled some morphological similarities with *A. nepalicus* and *A. marmoratus*, it is genetically different from topotypic *A. marmoratus* and *A. nepalicus*. Possibly, *A. marmoratus* does not occur in Nepal. Whereas, the *Amolops* population from Kimathanka, Sankhuwasava and Lamatar, Taplejung showed similar morphological characteristics of holotype of *A. formosus* (BMNH1947.2.4.18, formerly 1874.4.17.281) and identified as *A. formosus* (Table 7).

All *Amolops* species in the Mahabharat range of Nepal were originally identified as *A. marmoratus* based on morphology. This method has resulted in misleading the species identification, especially for the cryptic taxa like *Amolops* which possess high

morphological similarities (Bain *et al.*, 2003; Lyu *et al.*, 2019b). In the recent years, with the advancement of molecular techniques, several cryptic amphibians have been identified as new species from Nepal (Khatiwada *et al.*, 2015; Khatiwada *et al.*, 2017; Mahony *et al.*, 2018; Khatiwada *et al.*, 2019a). This study has resolved previous taxonomic confusion in the genus *Amolops* using molecular phylogenetic analysis. The discovery of *A. mahabharatensis* sp. nov. may indicate the presence of additional cryptic diversity within *Amolops* species from Nepal.

Natural history and conservation: Majority of people in the survey area have a strong belief in ethnoherpetology. Ethnic communities in the mountainous areas of Nepal depend on natural resources for their livelihood (Khatiwada and Haugaasen 2015). They engage in the collection of tadpoles, adults and even eggs of *Amolops* species. Khatiwada and Haugaasen (2015) also reported the overharvesting of *Amolops* species. The amphibian population is highly threatened and at the risk of extinction by recent global environmental changes and further accelerated by anthropogenic activities like overharvesting (Chan *et al.* 2014). Since, the richness and abundance of *Amolops* species is relatively low compared to other amphibian species in Nepal (Khatiwada *et al.*, 2019b), monitoring of the populations of edible frogs should be a conservation priority in the study area and elsewhere in Nepal.

Acknowledgements We are thankful to Subarna Ghimire, Suman Sapkota, Purnman Shrestha, Bibas Shrestha, Bijaya Paudel, Suman Acharya and Anish Timsina for their assistance during fieldwork. We are grateful to the Department of National Park and Wildlife Conservation (DNPWC) and Department of Forest, Government of Nepal for providing necessary research permit for conducting the research. We are

Table 7 Measurements (in mm; mean±SD) of *Amolops nepalicus*. See Material and methods for abbreviations.

Abbreviation	Female										Male										Mean ± SD
	CDZMTU 0133	CDZMTU 0137	CDZMTU 0140	CDZMTU 0141	CDZMTU 0142	CDZMTU 0134	CDZMTU 0135	CDZMTU 0136	CDZMTU 0138	CDZMTU 0139	CDZMTU 0143	CDZMTU 0144									
SVL	79.08	51.02	78.84	45.34	45.94	60 ± 15.6	38.36	41.86	46.67	45.71	35.67	37.6	42.02	41.1 ± 4.1							
HL	28.98	16.04	27.93	15.11	14.9	20.6 ± 6.4	11.22	12.22	12.54	15.46	12.22	12.7	13.84	12.9 ± 1.4							
HW	27.58	17.86	25.2	14.97	14.96	20.1 ± 5.3	11.74	13.26	14.59	13.49	12.87	11.99	14.62	13.2 ± 1.1							
SL	4.12	3.91	6.46	3.13	4.05	4.3 ± 1.1	1.36	3.8	3.62	2.93	2.62	2.94	3.53	3.0 ± 0.8							
ED	8.61	6.72	9.79	6.4	5.9	7.5 ± 1.5	3.36	4.85	5.19	6.56	4.92	4.51	5.67	5.0 ± 1.0							
IND	6.03	2.98	5.81	2.57	3.06	4.1 ± 1.5	2.56	2.17	2.89	2.86	2.09	2.14	2.32	2.4 ± 0.3							
ENL	6.16	4.21	4.75	4.13	4.33	4.7 ± 0.8	4.52	3.4	3.7	3.42	1.8	2.68	4.21	3.4 ± 0.9							
UEW	5.25	5.77	9.85	3.49	4.33	5.7 ± 2.2	4.53	2.82	5.13	3.4	3.39	3.17	3.3	3.7 ± 0.8							
IOW	7.46	5.25	8.1	4.43	5.21	6.1 ± 1.4	4.59	4.19	3.53	4.55	5.08	4.93	4.72	4.5 ± 0.5							
TYD	2.92	2.23	3.29	1.42	1.75	2.3 ± 0.7	1.64	2.96	1.36	1.39	2.18	1.47	1.91	1.8 ± 0.6							
FAL	16.21	8.67	17.49	9.91	7.98	12.1 ± 4	8.07	8.97	9.89	9.51	8.09	7.39	8.84	8.7 ± 0.9							
HAL	42.5	23.47	38.4	23.21	19.57	29.4 ± 9.2	20.02	18.21	22.82	20.57	18.38	15.72	21.07	19.5 ± 2.3							
F1	3.86	4.44	6.7	2.78	3.09	4.2 ± 1.4	1.86	3.17	2.92	2.7	1.78	2.54	2.94	2.6 ± 0.5							
F2	3.85	5.25	7.57	4.17	3.76	4.9 ± 1.4	3.55	3.35	2.29	3.68	2.87	2.58	3.32	3.1 ± 0.5							
F3	4.91	4.62	6.06	4.81	6.14	5.3 ± 0.7	3.7	5.99	2.8	3.65	3.43	3.17	6.39	4.2 ± 1.4							
F4	4.19	5.42	6.07	4.23	4.26	4.8 ± 0.8	2.8	4.97	4.37	2.49	4.49	4.01	6.01	4.2 ± 1.2							
THL	44.95	27.99	48.2	25.67	20.98	33.6 ± 10.9	23.9	20.58	17.67	20.94	21.13	21.55	22.61	21.2 ± 1.9							
SHL	53.31	32.21	54.96	27.69	21.7	38 ± 13.6	23.47	26.41	17.77	25.69	20.06	22.81	25.56	23.1 ± 3.2							
TL	27.54	15.34	27.01	14.55	12.57	19.4 ± 6.5	12.59	14.08	11.64	13.11	11.77	12.18	13.43	12.7 ± 0.9							
FOL	41.59	26.28	39.52	22.31	20.28	30 ± 8.9	16.83	23.24	17.21	19.43	16.77	18.26	19.02	18.7 ± 2.3							
T1	6.31	2.27	6.63	3.48	3.45	4.4 ± 1.7	2.71	3.15	2.18	2.67	2.65	3.23	2.64	2.7 ± 0.4							
T2	8.93	2.5	8.68	4.95	4.25	5.9 ± 2.5	3.34	4.95	2.21	4.11	4.02	3.55	3.9	3.7 ± 0.8							
T3	6.83	2.25	7.2	3.85	5.91	5.2 ± 1.9	3.34	4.26	4.06	2.98	6.01	6.24	3.12	4.3 ± 1.3							
T4	9.15	7.73	7.89	4.6	9.74	7.8 ± 1.8	3.5	5.01	5.15	3.53	10.31	8.86	4.27	5.8 ± 2.7							
T5	8.53	5.24	7.75	4.6	5.95	6.4 ± 1.5	4.16	4.29	2.26	3.53	6.78	7.84	3.99	4.7 ± 1.9							

Table 8 Measurements (in mm; mean±SD) of *Amolops formosus*. See Material and methods for abbreviations.

Abbreviation	BMNH 1947.2.4.18	CDZMTU 0145	CDZMTU 0146	Mean ± SD
	Female	Female	Female	
SVL	63.12	73.14	69.84	68.7 ± 5.1
HL	20.54	13.94	9.59	14.7 ± 5.5
HW	21.53	20.83	17.61	20 ± 2.1
SL	7.96	6.18	5.17	6.4 ± 1.4
ED	7.18	8.08	6.79	7.4 ± 0.7
IND	6.72	6.62	5.53	6.3 ± 0.7
ENL	4.02	2.9	5.16	4 ± 1.1
UEW	4.35	3.9	2.87	3.7 ± 0.8
IOW	6.17	6.62	4.69	5.8 ± 1
TYD	2.14	2.83	1.97	2.3 ± 0.5
FAL	17.72	8.99	9.04	11.9 ± 5
HAL	22.08	34.1	34.89	30.4 ± 7.2
F1	8.78	6.01	10.9	8.6 ± 2.5
F2	11.75	10.11	12.17	11.3 ± 1.1
F3	18.59	16.5	17.6	17.6 ± 1
F4	13.85	9.02	12.52	11.8 ± 2.5
THL	33.47	35.82	33.88	34.4 ± 1.3
SHL	35.97	36.58	38.96	37.2 ± 1.6
TL	18.07	29.1	15.21	20.8 ± 7.3
FOL	36.34	21.18	16.77	24.8 ± 10.3
T1	13.28	8.78	10.18	10.7 ± 2.3
T2	14.26	12.7	13.45	13.5 ± 0.8
T3	23.69	19.12	18.61	20.5 ± 2.8
T4	32.07	28.94	28.96	30 ± 1.8
T5	22.56	23.7	23.55	23.3 ± 0.6

thankful to Benjamin Tapley, Zoological Society of London, London, UK for the measurement of holotype of *Amolops formosus*, reviewing English language and also provided critical comments and suggestions that substantially changed the quality of the manuscript. We are thankful to two anonymous reviewers and Prof. S. D. Biju, University of Delhi for their insightful comments, suggestions and improvements in the final version of manuscript. This work was supported by the Second Tibetan Plateau Scientific Expedition and Research Program (STEP), Grant No. 2019QZKK04020202 and National Natural Sciences Foundation of China (NSFC-31471964) granted to Jianping Jiang. Janak Raj Khatiwada was supported

by Chinese Academy of Sciences President's International Fellowship Initiative (2018PB0016).

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Handling Editor: Chen YANG

How to cite this article:

Khatiwada J. R., Shu G. C., Wang B., Zhao T., Xie F., Jiang J. P. Description of a New Species of *Amolops* Cope, 1865 (Amphibia: Ranidae) from Nepal and Nomenclatural Validation of *Amolops nepalicus* Yang, 1991. *Asian Herpetol Res*, 2020, 11(2): 71–94. DOI: 10.16373/j.cnki.ahr.190052

Appendix

Table S1 Specimens used for morphological comparisons. CIB refers to Chengdu Institute of Biology, and BMNH to The British Museum of Natural History, UK.

Species name	Locality	Museum collection number	
		Male	Female
<i>A. formosus</i>	Assam, India		BMNH1947.2.4.18
<i>A. afghanus</i>	Yunnan, China	CIB33428	
<i>A. chunganensis</i>	Sichuan, China	CIB105439 CIB105440 CIB105442	CIB105440 CIB125942
<i>A. medogensis</i>	Medog, Tibet, China	CIB97508 CIB97509	CIB97507 CIB97511 CIB97512
<i>A. daiyunensis</i>	Mt. Daiyu, Fujian, China	CIB33594 CIB33595	
<i>A. granulosus</i>	Sichuan, Chinaw	CIB84238 CIB93656	CIB84231 CIB84232 CIB84233 CIB3723 CIB3725 CIB3733 CIB3734
<i>A. hainanensis</i>	Hainan, China	CIB84160 CIB84161 CIB84315	
<i>A. lifanensis</i>	Sichuan China	CIB34118 CIB34120	
<i>A. mantzorum</i>	Sichuan China	CIB94975 CIB94984	CIB94925 CIB94976 CIB34035 CIB34029
<i>A. ricketti</i>	Shanghang, Fujian, China	CIB4087 CIB4097	CIB4094 CIB4098 CIB93633
<i>A. torrentis</i>	Hainan, China	CIB95425 CIB95435	CIB95427 CIB95436